OHIO UNIVERSITY TODAY

1980 - 1982

Spec. Coll.
Ref
LH1
O55
O552x

Ohio University
TODAY
Fall 1981 11
A Special Report

University Team Announces First Transfer of Functioning Genetic Material from One Mammalian Species to Another

On September 8 a team of Ohio University molecular geneticists led by Thomas E. Wagner and Joseph D. Jollick announced a major scientific achievement, the first transfer of functional genes from one mammalian species to another.

The researchers had transferred hemoglobin (blood protein) genes from rabbits to mice, resulting in mice being born with the rabbit gene and producing rabbit hemoglobin.

The public at large and Ohio University alumni heard reports of the historic breakthrough, which was broadcast on television and radio and by print media throughout the world.

An official report of the study was published in the October issue of the *Proceedings of the National Academy of Science.* Before acceptance by the journal, it had been rigorously reviewed by leading scientists who noted its significance.

The team had accomplished their feat by microinjecting the genetic material directly into the male pronucleus, a sperm cell that has recently penetrated an egg and has swollen in preparation for combining with the female DNA.

Wagner, the originator of this route for introducing foreign DNA, believes it prohibits the egg from "recognizing" the rabbit gene as a totally foreign material.

"We're essentially tricking the egg into believing the rabbit DNA is part of the male mouse DNA that it must accept anyway," Wagner explained to *The Washington Post's* science editor, Victor Cohn.

Other researchers unsuccessfully attempting to introduce foreign genetic material into embryos have done so after the eggs had combined with the sperm. Wagner's decision to attempt gene transfer during the sperm pronuclear stage was based on eight years of study of embryo development: "We knew there had to be this short period of time in the history of an organism when it does naturally accept somewhat foreign genetic material," he said.

The success of this method opens new avenues for exploration: It's expected to short-cut the breeding of superior farm animals and suggests a potential means of curing human genetic disease. (See related story on future applications of the research.)

Publication of the research report was the formal announcement to the scientific community of findings first confirmed at Ohio University nine months ago by Wagner and Jollick and their Ohio University research associates David R. Scholl, Richard L. Hodinka and Janice A. Gault, a faculty member and immunologist.

Another team member, Peter Hoppe, of Jackson Laboratories in Bar Harbor, Maine, performed the microinjection. Hoppe had earlier collaborated with Karl Illmensee of the University of Geneva in research that achieved the first cloning of mammals (mice), a feat announced last January.

Hoppe requested that any reference to him be omitted from early announcement of the research and withheld until publication of the journal article. His cooperation with Wagner and Jollick was known within the scientific community, however, and the role of the publicity-shy scientist was revealed by some science writers.

Wagner, who had developed his idea for the successful transfer early in 1980, discussed it with Hoppe during a symposium in late summer and enlisted his collaboration during a New York taxi ride. Soon after, Wagner recruited Jollick, a member of the basic science faculty for the College of Osteopathic Medicine who holds a dual appointment in Arts and Sciences. The two colleges funded the Wagner-Jollick research.

A microbial geneticist, Jollick was responsible for the preparation and purification of the rabbit gene, which had been cloned in bacteria. Last November, the rabbit genes were shipped from Athens to Bar Harbor.

At his laboratory, Hoppe microinjected the genes into more than 300 mouse eggs. More than 200 survived the incubation period and were transferred to recipient mouse foster mothers, which produced 46 offspring.

The mouse pups were then returned to the University, and from that point on, Jollick and Wagner immersed themselves in hematological analysis.

In January, after a series of late nights, the researchers got their first positive evidence. Immunological data showed that five of the tiny pups contained rabbit hemoglobin protein in their red blood cells.

"We were very, very excited when we first saw that the mice were producing a rabbit hemoglobin," Wagner recalled. "Although that initial elation dissipated in the work that followed, another high came when we confirmed that a second generation of mice also contained the gene and produced rabbit hemoglobin."

In all, three major tests to confirm the gene transfer were conducted: The team showed immunologically that the rabbit gene product was present in the mice; they showed the presence of the rabbit DNA in the mouse tissues; and they demonstrated the presence of an additional protein in the red blood cells of the mice.

Several times the researchers were forced to "put on a full-court press" and take their work home. For a period, Jollick even used the oven in his kitchen to incubate materials. He recalled: "I'd find myself waking up with ideas in the middle of the night, so I began sleeping with a notepad by my bed so I could jot them down."

Following the confirming tests, Wagner wrote the paper in a concentrated six-hour effort. It was relayed to Dr. Elizabeth Russell, the National Academy of Science sponsor who, in turn, sent it out to reviewers. Following an additional study suggested by one of the reviewers, the paper was accepted for publication.

By the time that University alumni read this account, the breakthrough will have been reported again and again in newspapers and scientific magazines throughout the world.

Beginning with a September 8 report in the *Washington Post,* it was picked up by the wire services and the national networks. A morning press conference in Athens was followed by television-taping sessions and by telephone interviews with reporters from the London *Sunday Times,* the Toronto *Daily* Mail and other print media from San Francisco to Miami.

The hullabaloo surprised, touched and exhausted Wagner and his associates. Hours after the press conference, the weary researcher admitted his amazement: the response had been even greater than he'd expected.

LINDA SHAW

Dr. Thomas E. Wagner explains to reporters at a September 8 press conference the procedure used to transplant a rabbit hemoglobin-producing gene into a recently fertilized mouse egg. A greatly enlarged photograph of the microscopic egg is shown on the screen behind Wagner.

A day after the announcement, Wagner headed back to Denver where he's working during a year's leave of absence on agricultural applications of his research at Genetic Engineering Inc. The company, which now holds a license agreement with the University on commercial uses of the technology, also is supporting application of the gene-transfer technique under a three-year, $190,000 grant to the University.

The same day, Joe Jollick stayed in his lab in Irvine Hall, accepting only the most pressing telephone calls.

"I've got to get back to work," he said.

by Dinah Adkins

Ohio University
TODAY

LINDA SHAW

Dr. Joseph D. Jollick, left, and Dr. Thomas E. Wagner examine slides documenting steps in their experiment.

Breakthrough Could Lead to Genetically Improved Animals

by Dinah Adkins

The history-making announcement of the first transfer of functional genetic material between species holds out some near and some far-flung promises.

—First, it will likely lead to major developments in the animal breeding industry, allowing breeders to speed up the process of selection and to produce stronger and more efficient animals.

—Secondly, it suggests that human genetic "diseases" may be cured by introducing the right gene into the embryonic brew that is flawed by missing or distorted ingredients. More immediately, the new techniques could also prove useful in studying genetic disease by introducing flawed genes into animal hosts.

—Thirdly, and strangest of all, the research holds uut the possibility that new species may be developed in the future by geneticists, although not the "Mickey Cottontails" that became the butt of laboratory jokes here during the Wagner-Jollick experiments.

"Mixing" of species would likely involve only the introduction of a few foreign genes or gene sequences, according to Wagner. Still, traits that make wild animals hardy or that make them more efficient at converting natural forage to protein might be added to the genetic makeup of domestic animals.

Of these three possibilities, however, Wagner believes only the first is probably due to see major advancement within the next decade. Much more research is necessary before any attempts will be made to "cure" genetic diseases in humans, he says, stressing that the University team does not include medical doctors so it will not be involved in that effort.

Likewise, Wagner suggests that mixing species in other than preliminary demonstration projects such as his own is far down the road. "No one knows what the foreign genes are really doing there — whether they're functioning as they would under normal conditions," he says.

A breeder of Finn sheep and Belgian horses, the professor plans to dedicate a major portion of his future time to improving species of domestic animals. During the current academic year, he's on leave from the University to continue his research at Genetic Engineering Inc., a Denver-based company that specializes in developing new livestock breeding techniques. One of his tasks there is to develop "three-parent" cattle, something he says could have a major effect on animal husbandry within 10 to 20 years.

Working cross-country with fellow researcher Jollick, Wagner hopes to help point the way to an agricultural revolution comparable to the development of artificial insemination and the new embryo transfer techniques. Such techniques are allowing "super sires" and valuable cows to mate and produce thousands more offspring than would naturally be possible.

To get an idea of the amount of money involved in such technologies one should note that the newest, embryo transfer, is already reportedly a $25 million business, with an estimated potential of up to $1 billion in the meat and dairy industries in 10 years. Although no one knows what the worth of "three-parent" animals may be, gene splicing techniques in general are expected to result in $50 to $100 billion of business by the turn of the century to agriculture alone, according to *The New York Times*.

As Wagner explains future three-parent procedures, traits for size or efficiency could be amplified by introducing characteristics of a third parent into an embryo of already superior farmstock. And this can be done while bypassing the lengthy and tedious process in which these genes are selected through generations of breeding.

Also, geneticists need only choose the "good" gene they want to introduce into an embryo, safely ignoring the same animal's bad characteristics.

"For example, if you have a bull that has a single outstanding characteristic but otherwise is a mediocre animal, it should be possible to take the complex of genes that code for that outstanding characteristic and add it to an embryo resulting from animals that are outstanding otherwise but lacking in this trait—without introducing the third parent's inferior qualities."

According to Wagner, an important contribution of animal husbandry could be to breed animals that are more efficient under poor feed conditions, even producing well on natural forage alone.

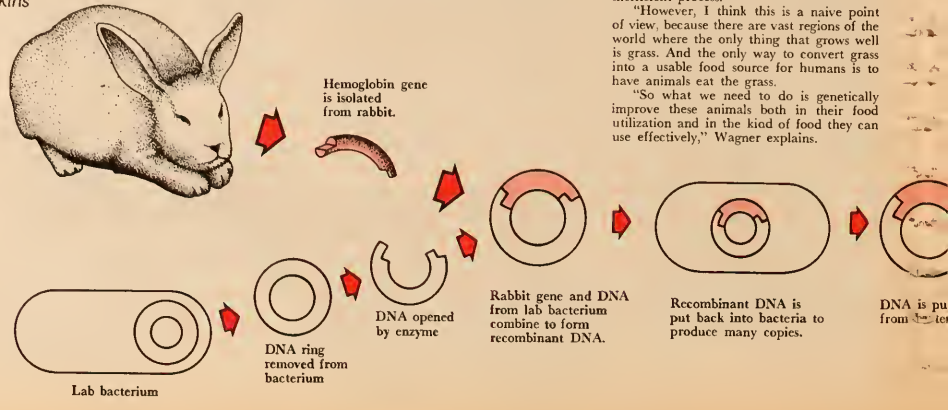
"So you might take a cow suited for growth in a sub-Saharan climate and increase its milk production dramatically, essentially making it more efficient in converting grass into protein that is usable by humans," the professor explains.

That could help alleviate the world food shortage, he speculates.

"There are those who believe that in order to meet the oncoming food shortage we should turn away from the animal side of agriculture and just grow plants. This is because raising crops to feed them to animals which could produce protein is an inefficient process.

"However, I think this is a naive point of view, because there are vast regions of the world where the only thing that grows well is grass. And the only way to convert grass into a usable food source for humans is to have animals eat the grass.

"So what we need to do is genetically improve these animals both in their food utilization and in the kiod of food they can use effectively," Wagner explains.



The professor also suggests that it may be possible to introduce an entire extra chromosome, creating an XYY bull for example. In this manner, animal breeders might be able to control some of the less desirable sex-linked traits in beef cattle.

Wagner believes these techniques are no different than those that created the dairy cows that currently produce two and a half times as much milk as they did 30 years ago. The new techniques are only more advanced and have speeded agriculture farther down the road toward the ultimate "factory animal," he says.

Herds of three-parent animals will probably be in existence within the next 10 to 20 years, Wagner believes, noting that the difficulty will not be in pooling genes but identifying which gene sequences correlate with which traits. Only a handful of genes have been identified in all species and almost none in cattle.

"That's one of the things we'll be working on," he explains, "the mapping and cloning of genes of interest."

The molecular geneticist warns that the more breeding is speeded up to obtain more specialized animals, the greater is the need to maintain herds of diverse types of cattle, or at least frozen embryos. The danger is that the gene pool, which has already become less diverse through selective breeding techniques, might suffer further. And everyone agrees that diversity is strength, making it more likely that animals can evolve defenses against climatic changes and other hazards to survival.

Wagner explains that most dairy cattle today are Holsteins, the most efficient animals under rich feed conditions. But other breeds, Ayrshires and Jerseys, for example, are more efficient under poor feed conditions.

"It's terribly important to keep the gene stocks of these other breeds," he stresses. "If we make mistakes, we may want to go back."

The Research Team Members

The leader of the research effort, Dr. Thomas E. Wagner, 38, conceived the idea of pronuclear injection of foreign DNA early in 1980. He worked on all phases of the project and is the senior author of the paper describing the experiment.

His decision to introduce foreign genetic material early in the sperm pronuclear stage was the result of earlier studies of mammalian fertilization processes. Other work by Wagner has shed light on gene regulation in general and—specifically—on the regulation of viral genes.

In 1980, Wagner became the first person to chemically switch a cancer-causing virus on and off. Published in *The European Journal of Biochemistry,* that experiment was termed "one of the most significant advances in molecular biology in the last decade" by Dr. Thomas C. Spelsberg of the Mayo Clinic.

Wagner earned his Princeton undergraduate degree magna cum laude at age 21 and earned his doctorate two years later from Northwestern University. From there he moved to the Cornell University Medical School faculty and the staff of the Sloan-Kettering Memorial Cancer Research Center.

A native Ohioan, he made a personal decision to leave New York City in 1969, preferring a rural setting. He came to Athens in 1970 as an assistant professor and rose rapidly to become a full professor in 1978.

He lives with his wife Susan and three children on a 60-acre farm where he breeds Finn sheep and Belgian horses. His interest in farming was inspired by childhood summers spent on his maternal grandfather's Ohio farm.

Dr. Joseph D. Jollick, '40, a microbial-geneticist on the basic science faculty supported by the College of Osteopathic Medicine, joined the research team in September 1980. Jollick's role included the purification of the rabbit genetic material and preparation of it for microinjection. Following implantation of the embryos and the birth of mouse pups, Jollick was involved in extensive hematological analysis of them.

With a bachelors degree from California State College in Pennsylvania and a masters from the American University in Washington, D.C., Jollick earned his PhD in medical microbiology from West Virginia University in 1969. He was awarded a National Research Council postdoctoral grant to work in the U.S. Department of the Army laboratories at Frederick, Md., where he focused on *Staphyloccocus* genetics.

Jollick was on the faculty of Wayne State University's School of Medicine and developed the gene transfer system for the *Caulobacter* bacteria there before coming to Athens in 1972. A native of a small town near Uniontown, Pa., he, like Wagner, prefers Southeastern Ohio's rural setting to city life. He lives with his wife Sandra and two sons on an Athens County farm.

Janice Gault, MS '71, joined the zoology and microbiology faculty in 1972. On the project, she worked on immunological and hematological testing of the original microinjected mice and their offspring to ascertain the presence of rabbit hemoglobin.

"It was a once-in-a-lifetime experience," she says of the research breakthrough, and adds that she was not surprised at the excitement and recognition that followed announcement of the project's success: "Looking at what science recognizes as highpoints or major events, it was reasonable to believe that this was in that category," she says.

Now 52, Mrs. Gault worked as a medical technologist after her marriage, and "in between and around four children" managed to earn her advanced degree. She teaches a physiological chemistry laboratory and a course in histological techniques, is coteacher for the immunology course for both undergraduates and medical students and supervises the department's teaching associates in the introductory courses.

FRED NELSON

Five mice born from the microinjected one-cell embryos today have genes producing rabbit hemoglobin, and their offspring have inherited the gene.

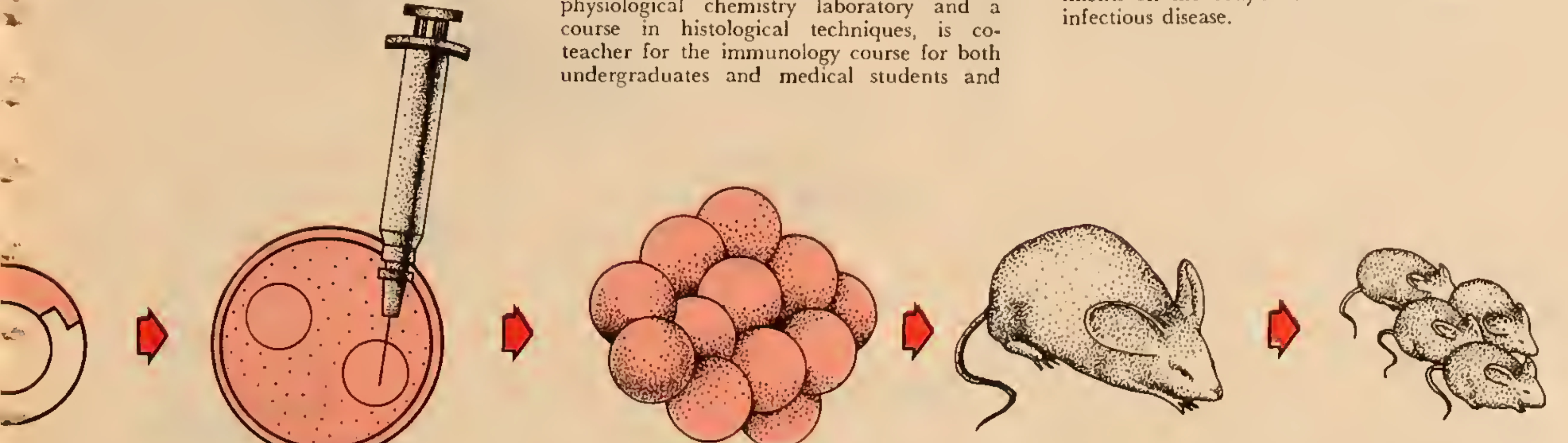
One of her children is a research assistant in the medical microbiology department at Ohio State University, but the others have bypassed science for careers as landscapers, guidance counselors and chefs.

Her husband, Jim, recently retired from his post as director of graduate admissions and advising.

David Scholl, PhD '81, was involved in the research process, Jollick says, "from the time the mice arrived right on through the very last test," adding that by the time the gene transfer project is completed, "Scholl will probably have about six publications to his credit."

After leaving campus, Scholl went on to the Roche Institute of Molecular Biology (after considering three offers) as a research associate. At Roche, he is involved in the study of the fundamental molecular biology of viruses and questions centered on the control of gene expression.

Richard L. Hodinka, a current PhD student, earned his master's degree from the University of Montana and his bachelor's at Marietta College. As a member of the team, he was involved in the preparation and examination of the mouse hemoglobin. His current work is with microbiologist Malcolm Modrzakowski, a Jollick colleague, on experiments on the body's defense mechanism to infectious disease.



purified
teria.

DNA is injected into one-cell mouse embryo.

Mouse embryo is cultured and divides.

Embryo is implanted in pseudo-pregnant foster mother mice.

Mouse litters are born, examined and found to contain both gene and the hemoglobin it produces.

Ohio University
TODAY

Graduate and Undergraduate Students Contribute to the Ongoing Research

University scientists are not lonely figures at work in their labs, but more aptly described as heads of teams that include undergraduate, graduate and post-doctoral students.

Dr. Wagner and Dr. Jollick are no exception, and Wagner is quick to express pride in the students who have worked with him over the past 10 years on campus.

"All of the work that had gone on in my lab prior to the interspecies gene transfer experiment was in some way related to that project, and students have been very influential and important in the ideas they have contributed and the work they have done to develop the research," he says.

Wagner names 13 students who have been associated with him or are now in his lab:

—Dexter Moore, PhD '74, is a physical biochemist who went on to postdoctoral research studies at Berkeley and then joined the chemistry faculty of Temple University.

—Vaughn Vandegrift, PhD '74, is a tenured associate professor at Murray State University, and for the past several years has published five scientific papers per year.

—Martin Serra, PhD '76, is an associate professor of biochemistry at Allegheny College, where he is an outstanding undergraduate teacher.

—Marilee Sipski, PhD '76, went on for her medical degree from Ohio State University and is a resident in pathology at the University of Minnesota headed for a career in research medicine.

—William Efcavitch, PhD '78, went on for three years of postdoctoral work at the University of Colorado, where he was an important part of a team which developed a new technique for the automated chemical synthesis of genes. The technique allowed for the development of a new piece of equipment, commonly known as the "gene machine." Efcavitch helped found and is now a partner in Amgene, a San Francisco firm marketing these instruments.

Bennett Cohen, PhD '79, spent a year as a postdoctoral research associate at the Roche Institute of Molecular Biology at Nutley, N.J., and then was invited to join the permanent senior staff, "perhaps the only postdoctoral associate to have directly joined the staff in the past 10 years," according to Wagner.

—Christine Shewmaker, PhD '79, received a Jane Coffin Childs Fellowship for two years of international postdoctoral study. She used the award to study recombinant genetic research on the mechanism of the expression of the hemoglobin gene with Dr. Richard Flavell at the National Institute for Medical Research in London. She is now back on campus as a postdoctoral research associate in microbiology and biochemistry.

—Chung Ho Chang is presently a doctoral student working in Wagner's lab and is involved in the continuation of the study of the control of the expression of the SV 40 tumor virus.

Wagner also named five undergraduates who, he noted, "contributed greatly to our research efforts":

—Joseph Harford '73 went on to earn a PhD in biochemistry from John Hopkins University.

—Deborah Skafar '77, an Honors Tutorial College graduate, went on to work on a PhD in molecular biology from Vanderbilt University.

—Carolyn Breitenberger '76 went on for her PhD in molecular biology at the University of North Carolina.

—Philip Pellett '80, an Honors Tutorial College graduate, is presently working toward a PhD in virology and molecular biology at the University of Chicago.

—Bruce Patterson, a senior in the Honors Tutorial College from Winston-Salem, N.C., is currently working with Wagner and Jollick on continuing experiments in embryonic gene transfer between species.

Dr. Jollick is just as enthusiastic as Dr. Wagner about outstanding students such as Bruce Patterson:

"He's a botany major who came to the lab to seek us out. I told him he was welcome to work, and he puts in about 15 hours a week in addition to carrying 20 hours of course work. He's one of the authors on the paper I'm now working on, and by the time he graduates, he'll be listed as a coauthor on three journal articles."

In addition to David Scholl and Richard Hodinka, members of the gene transfer team, Jollick singles out PhD student David (Ta-Chin) Hua, who earned his degree in 1980 and went on for postdoctoral work at the University of Pennsylvania Medical School. Hua is now a visiting fellow at the National Cancer Institute, working on human lung tumor associated proteins.

A Worldwide Story

As an experienced political pundit recently observed, "When the syndicated cartoonists take on a subject, you know it's a national issue."

So it was with the genetic transfer report. The Stayskal cartoon, syndicated by the *Chicago Tribune*, appeared in subscribing papers a week following the announcement. Another, syndicated by the Copley News Service, focused on the surprise a rabbit felt upon reading of the experiment.

The cartoons were part of the ripple effect of the announcement which had been made by the influential science writer of the *Washington Post*, Victor Cohn. Following the early editions of the *Post*, the wire service reported the breakthrough—initially from Cohn's report and later from their own early morning interviews with Jollick and Wagner.

The major networks carried the report throughout the day and both CBS and Cable News Network had crews on campus to film and interview the scientists in their laboratory. Boston's Channel 4 followed up the next day with an interview with an MIT geneticist who interpreted the significance of the successful experiment.

Newspapers in every major city in the country carried the story and in the cattle-oriented West it frequently appeared on page one. It also was carried on the front page of the *International Herald Tribune*.

Editorial comment from Corpus Christi to Akron to Louisville spoke of the potential contributions to come from the transplant procedure, both for studying human disease and for breeding superior agricultural animals. In all, more than a thousand newspapers ran news and comment.

A second wave of interview requests came from science magazines and the international press. Subsequent reports appeared in such U.S. publications as *Science*, *Science News* and *Discover*. Reports abroad appeared in France's *Science et Vie*, Italy's *Panorama*, and *Somos* in Argentina.

But for the scientific community, the important publication was *The Proceedings of the National Academy of Science*. It was the October issue of this prestigious publication that carried the paper by Wagner and his colleagues describing the transplant procedure and the confirming immunological tests. This was the report that would be read and discussed by scientists for months to come.

"THAT'S THE FELLOW WHO DISCOVERED HOW TO TRANSFER RABBIT GENES TO MICE. I HEAR HE'S WORKING ON ANOTHER EXPERIMENT BUT WON'T SAY WHAT!"

Reprinted by permission of the Chicago Tribune-New York News Syndicate Inc.